GLYCOGEN METABOLISM OF ISOLATED RAT LIVER PERFUSED WITH LONG-CHAIN FATTY ACID

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1. Introduction

Struck et al. [1] reported that oleate stimulates glucose formation from lactate in the perfused liver. This observation was confirmed in several reports [2–4]. Further, gluconeogenesis from pyruvate [3,5,6] and alanine [7,8] has been observed to be stimulated by fatty acids. However, Exton and Park [9] observed only minimal effects on gluconeogenesis by oleate. Further, they suggested that the stimulation of glucose formation by long-chain fatty acids observed by others is secondary to an increased liver adenosine 3′,5′-monophosphate (cAMP) concentration. This conclusion was reached because fatty acids stimulated glycogenolysis in their experiments. Because of the importance of the observations and the suggestions of Exton and Park [9], glycogen metabolism of livers perfused with long-chain fatty acids was further investigated.

2. Materials and methods

The apparatus and technique of perfusion were as previously described [1,3,5]. Krebs-Henseleit buffer was substituted for Tyrode solution in the perfusion medium (100 ml) to give a final HCO_3^- content of 25 mM and a pH of 7.4 [10]. In all experiments to be reported, the medium used for the perfusion of the liver during the operation and transfer to the perfusion system contained 2.5 mM glucose.

After 40 min of pre-perfusion, a dose of 110 μmoles oleate emulsion and 100 μmoles DL-carnitine-HCl was given; and an infusion of 100 μmoles of oleate per hour was continued throughout the perfusion. The oleate emulsion was prepared in a solution of 12% bovine serum albumin (Behringwerke, Marburg, Germany) in Krebs-Henseleit buffer using a high-speed homogenizer. The control received carnitine and albumin in a manner similar to the oleate perfusion. Medium samples were taken at 10 min intervals and were analyzed as previously described [5]. Liver samples were taken by the rapid freezing technique of Wollenberger, Ristau and Shoffa [11]. Changes in liver glycogen were recorded in the same liver by taking samples at 40 min, before oleate, and then at 60 and 80 min after addition of oleate or control solutions. Liver glycogen was measured as described previously [12] and expressed as μmoles glucose/g wet weight.

3. Results

Perfusion of livers from fed rats with a medium containing 13.3 mM glucose maintained a constant liver glycogen content throughout the 40 min experimental period (table 1). Liver glycogen content after oleate addition (60 or 80 min) was similar to the levels before fatty acid administration (40 min).

Glycogen is synthetized by the perfused liver of fasted rats when the medium contains a high concentration of glucose (13.3 mM). The rates of glycogen synthesis were similar in the presence or absence of oleate (table 2). Decreases in perfusate lactate and pyruvate concentrations were observed with livers from fed or fasted rats perfused with oleate in the

Table 1

Glycogen content of perfused liver from well-fed rats in the presence of oleate. Livers were perfused as described in section 2 with 100 ml medium containing 13.3 mM glucose. Oleate (110 μmoles) and 100 μmoles of DL-carnitine-HCl were given at 40 min after a control liver biopsy was obtained. An infusion of 100 μmoles/h oleate was continued throughout the experiment. Results are given as mean ± S.E.M.

Additions	No. of observations	Liver glycogen (μmoles/g wet weight)		
		Before addition 40 min	After addition	
			60 min	80 min
None	4	241 ± 30	216 ± 19	230 ± 22
Oleate	4	222 ± 15	233 ± 14	230 ± 16

Table 2

Glycogen synthesis of perfused livers from fasted rats in the presence of oleate. The experiment was carried out as in table 1 except that the rats were fasted for 24 to 30 h before perfusion. Results are given as the mean ± S.E.M. Rate of glycogen synthesis is calculated from the increase in glycogen content from biopsies taken at 40 and 80 min.

Additions	No. of observations	Liver glycogen (μmoles/g wet weight)			Rate of glycogen synthesis (μmoles/g/min)
		Before addition 40 min	After addition		
			60 min	80 min	
None	3	10.2 ± 2.3	16.4 ± 3.6	18.5 ± 3.5	0.21 ± 0.03
Oleate	5	13.7 ± 2.2	18.1 ± 4.1	21.1 ± 2.2	0.19 ± 0.02

Table 3

Glycogen and total carbohydrate synthesis from lactate in perfused livers of fasted rats in the presence of oleate. Rats were fasted for 24 to 30 h before perfusion. Livers were perfused with 100 ml medium containing 2.5 mM glucose. Lactate (10 mM) was added after 40 min of pre-perfusion, and oleate was added as described in table 1. The rate of total carbohydrate synthesis was calculated from the increases in glycogen (40–80 min) and from the linear increase in the medium glucose. Results are given as the mean ± S.E.M.

Additions	No. of observations	Liver glycogen (μmoles/g wet weight)			Rate of total carbohydrate synthesis (μmoles/min/g)
		Before addition 40 min	After addition		
			60 min	80 min	
Lactate	4	3.5 ± 1.2	6.2 ± 1.8	6.3 ± 2.2	0.93 ± 0.10
Lactate + oleate	4	4.9 ± 1.8	6.8 ± 3.1	9.0 ± 3.3	1.65 ± 0.05

presence of 13.3 mM glucose, indicating a net uptake of these compounds in the presence of fatty acids. The increase in glycogen was slightly higher in livers perfused with oleate in addition to lactate in the medium (table 3). This is probably due to the fact that the rate of gluconeogenesis is stimulated (75%) by oleate which results in a higher medium glucose concentration (6.65 ± 0.26 mM) as compared to the control (5.68 ± 0.36 mM).

4. Discussion

Our present findings do not lend support to the suggestion of Exton and Park [9] that emulsions of free fatty acids lead to an increased liver cAMP level. It is generally accepted that increased liver cAMP level [13], either arising from the cAMP administration or from glucagon or epinephrine stimulation of adenyl cyclase [14], results in activation of phosphorylase with a stimulation of liver glycogenolysis and glucose release. But, in fact, the normal liver glycogen level characteristic of the fed liver remained constant even in the presence of oleate, which would not be the case if glycogenolysis had been stimulated by an elevated liver cAMP level. Furthermore, glycogen synthesis, in rates similar to those given in the literature for perfused livers of fasted rats [2,9,16] was not decreased by oleate addition. In actuality, oleate increased the liver glycogen deposition in livers perfused with physiological levels of glucose (6 mM) as a result of its stimulation of gluconeogenesis from lactate. This is in accordance with earlier findings in this laboratory [1].

A further observation that does not support the suggestion that fatty acids increase cAMP concentration is the fact that endogenous gluconeogenesis and urea production are stimulated by dibutryl cAMP and glucagon [3,12] while fatty acids have little effect on these processes.

The importance of fatty acid oxidation and endogenous liver fat mobilization in gluconeogenesis has been recently demonstrated [4, 15].

5. Summary

A stable liver glycogen content was maintained in isolated, perfused livers of fed rats even with oleate in the perfusate. Other experiments indicated that glycogen synthesis in livers taken from fasted rats remains unimpaired in the presence of oleate. These findings do not support the suggestion of Exton and Park [9] that stimulation of glucose formation by long-chain fatty acids is secondary to an increased liver cAMP level.

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